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ORIGINAL ARTICLE

Metabolism of sumatriptan revisited

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Abstract

Scientific literature describes that sumatriptan is metabolized by oxidative deamination of its dimethylaminoethyl residue by monoamine oxidase A (MAO A) and not by cytochrome P450 (CYP)-mediated demethylation, as is usual for such structural elements. Using recombinant human enzymes and HPLC-MS analysis, we found that CYP enzymes may also be involved in the metabolism of sumatriptan. The CYP1A2, CYP2C19, and CYP2D6 isoforms converted this drug into *N*-desmethyl sumatriptan, which was further demethylated to *N*,*N*-didesmethyl sumatriptan by CYP1A2 and CYP2D6. Otherwise, sumatriptan and its two desmethyl metabolites were metabolized by recombinant MAO A but not by MAO B to the corresponding acetaldehyde, with sumatriptan being only a poor substrate for MAO A compared to the *N*-demethylated and the *N*,*N*-didemethylated derivatives.

KEYWORDS
cytochrome P450, metabolism, monoamine oxidase, sumatriptan, zolmitriptan

1 | INTRODUCTION

About 50% of all drugs on the market have basic structural elements,[1] with dimethylaminoalkyl groups being very common. During the metabolism of such functionalities, found in antihistamines, antidepressants, opioid analgesics, local anesthetics, as well as other drug classes, preferentially the amine moieties are demethylated by cytochrome P450 (CYP) enzymes. In this process, first a hydroxylation of the methyl group and then a hydrolysis of the resulting hemiaminal takes place with the release of formaldehyde.[2] In a few cases, the dimethylaminoalkyl groups are deaminated by monoamine oxidases (MAO) A and B to form an aldehyde.[3–6] Sumatriptan, a selective serotonin 5-$HT_{1B/1D}$ receptor agonist commonly used for the acute treatment of migraine, is an example for this kind of metabolism. In the scientific literature, it is generally assumed that the degradation of its dimethylaminoethyl residue predominantly or exclusively takes place via the MAO A pathway (Figure 1).[7–10] This finding is mainly based on an in vitro study with human liver homogenate.[11] The acetaldehyde derivative produced by MAO A is further oxidized by aldehyde dehydrogenases or oxidases to an acetic acid, which is glucuronidated in a phase II reaction. In contrast, in case of the structurally related zolmitriptan it is reported that CYP-mediated demethylation occurs first followed by degradation of the produced *N*-desmethyl zolmitriptan by MAO A to an aldehyde intermediate.[8,9,12,13] These peculiarities in the published metabolism of sumatriptan prompted us to re-examine the metabolism of sumatriptan using recombinant MAO and CYP enzymes.

2 | MATERIALS AND METHODS

2.1 | Materials

Enzymes: MAO A (69 U/mg, 2.5 mg/0.5 ml, Product No. M7316) and MAO B (66 U/mg, 2.5 mg/0.5 ml, Product No. M7441) (Sigma Aldrich, Steinheim, Germany); human recombinant CYP enzymes: Corning

Abbreviations: CYP, cytochrome P450 enzyme; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; MAO, monoamine oxidase; MS, mass spectrometry; PBS, phosphate-buffered saline.

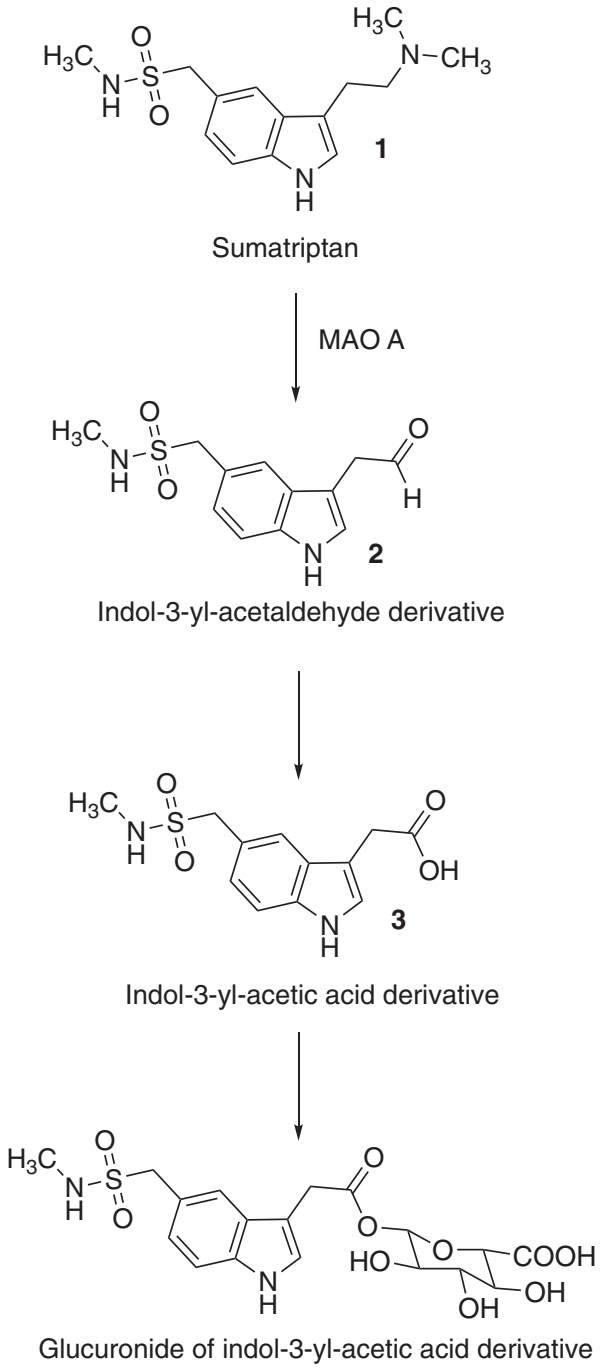

FIGURE 1 Metabolism of sumatriptan published in literature

Supersomes™ with oxidoreductase CYP1A2 (0.5 nM, Product No. 456203), CYP2C9 (1 nM, Product No. 456218), CYP2C19 (0.5 nM, Product No. 456219), CYP2D6 (0.5 nM, Product No. 456217), and CYP3A4 (1 nM, Product No. 456207) (Corning Live Sciences, Kaiserslautern, Germany). The enzyme preparations were divided into aliquots of 50 µl each and stored at −80°C until use.

Chemicals: Sumatriptan succinate, zolmitriptan (TCI Chemicals, Eschborn, Germany); *N*-desmethyl sumatriptan hemisuccinate, sumatriptan-*N*-oxide (Toronto Research Chemicals, Toronto, Canada); *N*,*N*-didesmethyl sumatriptan (AstaTech, Bristol, United States); phosphate-buffered saline (PBS) tablets (one tablet dissolved in 200 ml of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25°C), NH_4HCO_3, D-cysteine, nifedipine, omeprazole, phenacetin (Sigma Aldrich, Steinheim, Germany); $NADPHNa_4$ (Carl Roth, Karlsruhe, Germany); $MgCl_2$-hexahydrate (AppliChem, Darmstadt, Germany); acetonitrile (MS-grade) (Fisher Scientific, Schwerte, Germany); diclofenac sodium, propranolol hydrochloride (Caesar & Lorenz, Hilden, Germany); 4-(5-phenyl-2*H*-tetrazol-2-yl) butan-1-amine was synthesized by a published procedure.[14]

2.2 | Treatment of sumatriptan, *N*-desmethyl sumatriptan, *N*,*N*-didesmethyl sumatriptan, and zolmitriptan with MAO A and MAO B

Incubation procedure: A stock solution of the corresponding compound (10 mM) in DMSO was diluted with DMSO to the desired concentration for the experiments. To 5 µl of this dilution, 90 µl of PBS was added. Then, 5 µl of the MAO preparation was added and incubation was carried out at 37°C for 15 min or 60 min. The final concentration of the test compounds in 100 µl was 10 µM and 1 µM, respectively. The enzymatic reaction was stopped by treatment with acetonitrile (100 µl). After cooling on ice for 10 min, the samples were centrifuged at 12 000 × *g* and 10°C for 5 min. The supernatants obtained were transferred to HPLC vials and subjected to HPLC analysis. Control samples were prepared analogously, replacing the enzyme solution with the same amount of PBS buffer. To prove the activity of the enzyme solutions used, the known MAO A and MAO B substrate 4-(5-phenyl-2*H*-tetrazol-2-yl)butan-1-amine was incubated in the same manner at a concentration of 10 µM.

HPLC analysis: The released enzyme products were detected and quantified on a high pressure gradient HPLC/MS system from Shimadzu (LC-20ADXR HPLC-pumps, SIL-30 AC autosampler, LCMS-2020 single quad detector) (Shimadzu Corporation, Tokyo, Japan). The HPLC/MS system was operated with an ESI interface in positive single ion mode (SIM) with a capillary voltage of +4.5 kV. The MS conditions were optimized to a dry gas flow of 15 l/min, a nebulizing gas flow (nitrogen) of 1.5 l/min, a heat block temperature of 200°C and a desolvation temperature of 250°C. Aliquots of 2 µl were injected onto a Luna 3 µm C_8 column (3.0 × 150 mm) protected with a SecurityGuard™ Cartridge Phenyl (3.0 × 4.0 mm) (Phenomenex, Aschaffenburg, Germany). The autosampler temperature was 15°C and column oven temperature was set to 30°C. Gradient elution was used with solvent A (acetonitrile/10 mM aqueous $NaHCO_3$ solution, 10/90, v/v) and solvent B (acetonitrile/10 mM aqueous $NaHCO_3$ solution, 50/50, v/v) at a flow rate of 0.3 ml/min. Analysis of sumatriptan and its metabolites: 0–3 min: isocratic run at 10% B, 3–15 min: linear gradient to 90% B, 15–25 min: isocratic run at 90% B, 25–30 min: linear gradient to 10% B, 30–35 min: isocratic run at 10% B; analysis of the reference substrate 4-(5-phenyl-2*H*-tetrazol-2yl)butan-1-amine: 0–3 min: isocratic run at 10% B, 3–10 min: linear gradient to 95% B, 10–25 min: isocratic run at 95% B, 25–30 min: linear gradient to 10% B, 30–35 min: isocratic run at 10% B. To avoid contamination of the detector with components

of the enzyme preparation, the effluents of the HPLC column were directed to the MS detector only from minutes 4 to 25 using a divert valve. The analytes were detected as proton adducts. The metabolic stability of the parent compounds was calculated from their peak areas in the appropriate samples incubated with and without the enzyme.

2.3 | Derivatization of MAO A derived aldehyde with D-cysteine

Incubation procedure: A DMSO solution of sumatriptan, *N*-desmethyl sumatriptan, *N*,*N*-didesmethyl sumatriptan, zolmitriptan or the reference 4-(5-phenyl-2*H*-tetrazol-2-yl)butan-1-amine (0.20 mM, 5 µl) was incubated with MAO A for 60 min as described above. The final concentration of the test compounds in a volume of 100 µl was 10 µM. After 60 min, the samples were treated with an aqueous solution of D-cysteine (1 mM, 100 µl) and heated to 50°C within 5 min and at 50°C for further 10 min to derivatize the aldehyde produced by the enzyme to a thiazolidine-4-carboxylic acid derivative. Then acetonitrile (200 µl) was added and the samples were cooled on ice for 10 min and centrifuged at 12 000 × *g* and 10°C for 5 min. The supernatants obtained were transferred to HPLC vials and subjected to HPLC analysis. Control samples were prepared analogously, replacing the enzyme solution with the same amount of PBS buffer.

HPLC analysis: The HPLC/MS apparatus used was the same as described above. Aliquots of 2 µl were injected onto an Accucore aQ 2.6 µm column (2.1 × 100 mm) (Thermo Scientific, Darmstadt, Germany) protected with a SecurityGuard™ Cartridge Phenyl (3.0 × 4.0 mm) (Phenomenex, Aschaffenburg, Germany). The autosampler temperature was 15°C and column oven temperature was set to 30°C. Sumatriptan, *N*-desmethyl sumatriptan, *N*,*N*-didesmethyl sumatriptan, and zolmitriptan were analyzed using isocratic conditions (acetonitrile/water/formic acid, 10/90/0.1, v/v/v) at a flow rate of 0.2 ml/min with a run time of 20 min. For the reference 4-(5-phenyl-2*H*-tetrazol-2-yl) butan-1-amine gradient elution was applied with solvent A (acetonitrile/water/formic acid, 10/90/0.1, v/v/v) and solvent B (acetonitrile/water/formic acid, 90/10/0.1, v/v/v): 0–3 min: isocratic run at 10% B, 3–15 min: linear gradient to 95% B, 15–18 min: isocratic run at 95% B, 18–20 min: linear gradient to 10% B, 20–30 min: isocratic run at 10% B. To avoid contamination of the detector with components of the enzyme preparation, the effluents of the HPLC column were directed to the MS detector only from minute 2 or 3 to minute 20 using a divert valve. The MS detector was operated in the ESI-positive SIM mode and the analytes were detected as proton adducts.

2.4 | Treatment of sumatriptan, *N*-desmethyl sumatriptan, and zolmitriptan with CYP enzymes

Incubation procedure: To a solution of the appropriate compound in DMSO (0.50 mM, 2.5 µl) was added a solution of $MgCl_2$ in PBS (prepared by dilution of 0.50 ml of a 0.10 M aqueous $MgCl_2$ solution to 20 ml with PBS) (92.5 µl in case of CYP2C9 and CYP3A4; 87.5 µl in case of CYP1A2, CYP2C19, and CYP2D6). Then, a solution of the appropriate CYP enzyme preparation was added (5 µl in case of CYP2C9 and CYP3A4; 10 µl in case of CYP1A2, CYP2C19, and CYP2D6) followed by a solution of NADPH (15 mM) in above-mentioned $MgCl_2$/PBS solution (25 µl). The final concentration of the test compounds in 125 µl was 10 µM and that of NADPH 3 mM. The samples were incubated at 37°C for 60 min. Then the enzymatic reaction was stopped by treatment with acetonitrile (250 µl). After standing at room temperature for 10 min, the samples were centrifuged at 12 000 × *g* and room temperature for 5 min. The supernatants obtained were transferred to HPLC vials and subjected to HPLC analysis. Control samples were prepared analogously, replacing the NADPH solution with the same amount of $MgCl_2$/PBS solution (25 µl). To prove the activity of the enzyme preparations used, the specific CYP substrates phenacetin (CYP1A2), diclofenac (CYP2C9), omeprazole (CYP2C19), propranolol (CYP2D6), and nifedipine (CYP3A4) were treated in the same way with the corresponding CYP enzyme. The concentration of demethylated CYP product formed in the sumatriptan and *N*-desmethyl sumatriptan samples, respectively, was calculated with the aid of standard solutions (10 µM). These were prepared by adding $MgCl_2$/PBS buffer (122.5 µl) to a DMSO solution of *N*-desmethyl sumatriptan or *N*,*N*-didesmethyl sumatriptan (0.50 mM, 2.5 µl) and incubating the resulting mixture at 37°C for 30 min followed by the addition of acetonitrile (250 µl) and centrifugation at 12 000 × *g* and room temperature for 5 min.

HPLC analysis: The HPLC/MS system used was the same as described above. The investigation of the metabolism of sumatriptan, *N*-desmethyl sumatriptan and zolmitriptan was carried out as described above in the derivatization experiments with D-cysteine. HPLC analysis was performed in the same way with an Accucore aQ column, but using acetonitrile/water/formic acid (5/95/0.1, v/v/v) as eluent. For measuring the degradation of the CYP reference substrates (phenacetin, diclofenac, omeprazole, propranolol, nifedipine), aliquots of 2 µl were injected onto an ACE 3 µm C_{18} column (2.1 × 100 mm) (HiChrom, Berkshire, UK) protected with a SecurityGuard™ Cartridge C_{18} (3.0 × 4.0 mm) (Phenomenex, Aschaffenburg, Germany). The autosampler temperature was 15°C and column oven temperature was set to 30°C. The flow rate was 0.3 ml/min. Gradient elution was applied with solvent A (acetonitrile/water/formic acid, 10/90/0.1, v/v/v) and solvent B (acetonitrile/water/formic acid, 90/10/0.1, v/v/v): 0–3 min: isocratic run at 10% B, 3–15 min: linear gradient to 95% B, 15–18 min: isocratic run at 95% B, 18–20 min: linear gradient to 10% B, 20–28 min: isocratic run at 10% B. To avoid contamination of the detector with components of the enzyme preparation, the effluents of the HPLC column were directed to the MS detector only from minute 2.5 to minute 26 using a divert valve. The MS detector was operated in the ESI-positive SIM mode and the analytes were detected as proton adducts. The identity of the CYP metabolites *N*-desmethyl sumatriptan, *N*,*N*-didesmethyl sumatriptan, and sumatriptan *N*-oxide was additionally confirmed by HPLC with high-resolution MS. The measurements were carried out similarly on a Bruker (Bremen, Germany) micrO-TOF-Q II spectrometer with positive ion mode ESI.

3 | RESULTS

3.1 | Degradation of sumatriptan, *N*-desmethyl sumatriptan, and *N*,*N*-didesmethyl sumatriptan by MAO A and MAO B

Sumatriptan, *N*-desmethyl sumatriptan, and *N*,*N*-didesmethyl sumatriptan were incubated with MAO A and MAO B at concentrations of 10 μM and 1 μM for 15 min and 60 min, respectively. After terminating the enzyme reaction by the addition of acetonitrile and centrifugation, the amount of the test compounds was determined directly without further sample clean up by HPLC and single quad MS detection using a C_8 column and a gradient of acetonitrile and 10 mM aqueous $NaHCO_3$. The results obtained for the two different concentration levels did not differ significantly (Table 1). In the case of MAO A, the concentration of sumatriptan was only slightly reduced after 15 min and was somewhat above 90%. In contrast, the quantities of the two desmethyl sumatriptan derivatives were significantly more affected. The amount of *N*-desmethyl sumatriptan was diminished by about 20%, that of *N*,*N*-didesmethyl sumatriptan by about 30%. As expected, the conversion of the three compounds was much more pronounced after 1 hour of incubation. Now, about 25% sumatriptan, 50% *N*-desmethyl sumatriptan, and 85% *N*,*N*-didesmethyl sumatriptan were degraded. In contrast to MAO A, MAO B was not able to convert sumatriptan and its two desmethyl derivatives (≥99% stability, $n = 2$). Under the same conditions, a degradation of the structurally related zolmitriptan could not be detected by either MAO A or MAO B.

3.2 | Derivatization of the acetaldehyde formed by MAO A with D-cysteine

Since the expected metabolism product, the indol-3-yl-acetaldehyde **2**, could not be detected directly in the samples, an attempt was made to identify it by derivatization. For this purpose, sumatriptan, *N*-desmethyl sumatriptan, and *N*,*N*-didesmethyl sumatriptan were incubated at a concentration of 10 μM each for 60 min at 37°C and the samples were heated for 15 min at 50°C after the addition of D-cysteine. An alternative HPLC separation method was used for analyte detection. The stationary phase here was an Accucore aQ column, elution was carried out isocratically with a mixture of acetonitrile, water, and formic acid. In all three cases, the expected reaction product, the thiazolidine-4-carboxylic acid derivative **4** (Figure 2), could be clearly detected in the HPLC/MS spectra on the basis of the mass-to-charge ratio whereby the amount formed increased from sumatriptan to *N*-desmethyl sumatriptan and *N*,*N*-didesmethyl sumatriptan, respectively (Figure 3). The remaining amounts of sumatriptan, *N*-desmethyl sumatriptan, and *N*,*N*-didesmethyl sumatriptan were 84%, 35%, and 10% (n = 2) in these experiments and were about the same as those obtained in the experiments conducted without derivatization (79%, 48%, and 15%, Table 1). In case of zolmitriptan, an analogous thiazolidine-4-carboxylic acid derivative was not detectable in such derivatization experiments.

TABLE 1 Degradation of sumatriptan, *N*-desmethyl sumatriptan, and *N*,*N*-didesmethyl sumatriptan by MAO A

		Stability (%)[a]	
	Concentration	Incubation time	
Compound	(μM)	15 min	60 min
Sumatriptan	10	92 ± 6	79 ± 4
	1	91 ± 7	75 ± 3
N-Desmethyl sumatriptan	10	83 ± 7	48 ± 9
	1	81 ± 3	45 ± 5
N,*N*-Didesmethyl sumatriptan	10	63 ± 7	15 ± 2
	1	70 ± 7	13 ± 2

[a]Amount of sumatriptan and its desmethyl derivatives, respectively, remaining after incubation at 37°C; means ± standard deviations, n = 3; reference 4-(5-phenyl-2*H*-tetrazol-2-yl)butan-1-amine: stability 20 ± 2% after 15 min at 10 μM.[15]

3.3 | Metabolism of sumatriptan, *N*-desmethyl sumatriptan, and zolmitriptan by CYP enzymes

Sumatriptan and zolmitriptan were incubated in a concentration of 10 μM for 60 min in presence of NADPH with the five CYP enzymes that are mainly responsible for the metabolism of drugs, namely CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. The activity of the human recombinant enzymes used was previously tested with corresponding specific CYP substrates. These were completely or almost completely degraded (see Materials and Methods). Sumatriptan showed a slight but significant degradation by CYP1A2, CYP2C19, and CYP2D6. At the same time, the *N*-desmethyl product and, in the presence of CYP1A2 and CYP2D6, also traces of the *N*,*N*-didesmethyl product could be detected (Table 2 and Figure 4).

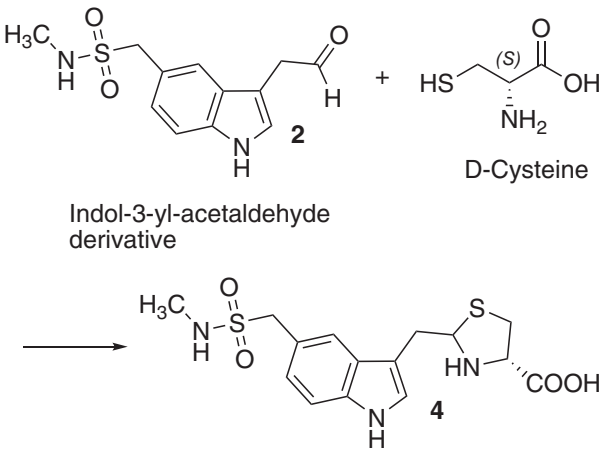

FIGURE 2 Formation of a thiazolidine-4-carboxylic acid derivative (**4**) from the MAO A generated aldehyde metabolite (**2**) of sumatriptan, *N*-desmethyl sumatriptan and *N*,*N*-didesmethyl sumatriptan, respectively, and D-cysteine.

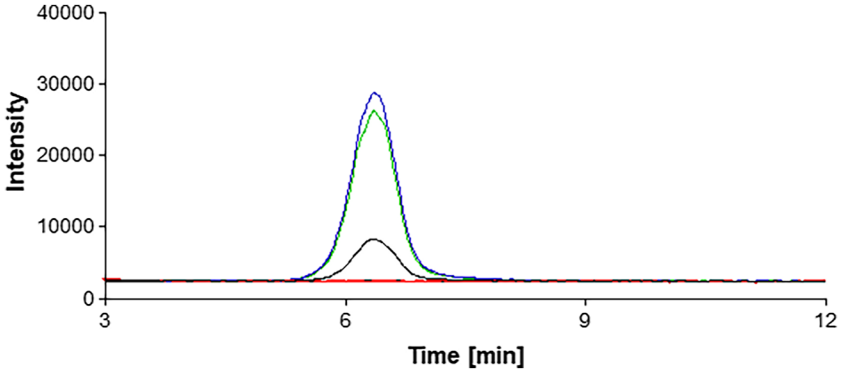

FIGURE 3 Superimposed RP-HPLC/MS chromatograms (single quadrupole, SIM, ESI+, *m/z* 370.10) of a sample of sumatriptan (black), *N*-desmethyl sumatriptan (blue), and *N*,*N*-didesmethyl sumatriptan (green) (10 μM each) after 60 min incubation at 37°C with MAO A and derivatization of the enzyme product (aldehyde **2**) with D-cysteine, and an analogue prepared control sample (red); column: Accucore aQ 2.6 μm, 2.1 × 100 mm, mobile phase: acetonitrile/water/formic acid (10/90/0.1, v/v/v); flow rate: 0.2 ml/min.

TABLE 2 Conversion of sumatriptan by different CYP enzymes

CYP Enzyme	Stability (%) of Sumatriptan[a]	Formation (%) of	
		N-Desmethyl sumatriptan[b]	*N*,*N*-Didesmethyl sumatriptan
1A2	92 ± 1	5 ± 1	Traces
2C9	≥99	–	–
2C19	95 ± 2	4 ± 1	–
2D6	87 ± 2	10 ± 1	Traces
3A4	≥99	–	–

[a]Amount of sumatriptan (initial concentration 10 μM) remaining after incubation at 37°C for 60 min in presence of NADPH.

[b]Quantity of *N*-desmethyl sumatriptan formed in relation to maximum possible amount. For determination, the appropriate reference compound was used.

Incubation of the *N*-desmethyl sumatriptan with the five CYP enzymes showed that degradation of this metabolite occurs only by CYP1A2 and CYP2D6, with the *N*,*N*-didesmethyl product being detected in each case (Table 3). In literature, it is described that after oral administration of sumatriptan to men also the *N*-oxide could be detected sporadically in plasma.[16] The formation of such kind of metabolites occurs primarily through flavin-containing monooxygenases (FMO), but can also be catalyzed in principle by CYP enzymes.[17] Indeed, after incubation of sumatriptan with CYP1A2 and CYP2D6, we detected small amounts of a compound with the retention time and the mass-to-charge ratio of the sumatriptan *N*-oxide reference substance (about 2% and 1%, respectively, of the maximum possible concentration). The identity of the *N*-oxide was further confirmed by HPLC with high-resolution mass spectrometry. In contrast, after incubation with CYP2C9, CYP2C19, and CYP3A4 the *N*-oxide was not detectable.

Zolmitriptan, like *N*-desmethyl sumatriptan, was metabolized by CYP1A2 and CYP2D6. A conversion by CYP2C19, as occurred in the case of sumatriptan, could not be detected (Table 4).

4 | DISCUSSION

The main metabolites of sumatriptan found in plasma after application of this drug in man are the indole acetic acid derivative and its acid glucuronide (Figure 1).[16,18–21] In in vitro studies with human liver preparations, MAO A was found to be the major enzyme involved in the oxidative deamination of the dimethylaminoethyl group of sumatriptan that precedes the formation of indole acetic acid.[11] Interestingly, this study did not provide any evidence for the involvement of CYP enzymes in phase I metabolism of sumatriptan. This is surprising, as normally dimethylaminoalkyl groups, which are frequently found in drugs, are metabolized by CYP via oxidative demethylation.[2] Direct oxidation of such groups to an aldehyde by MAO A has been described for some drugs, but occurs rarely overall.[3–6] We were therefore interested in whether sumatriptan can actually be metabolized initially only by MAO A or whether CYP enzymes are not after all able to degrade the dimethylamioethyl group, as has been described for the structurally related zolmitriptan.[8,9,12,13] This substance is reported to be first demethylated by CYP1A2, and only the resulting *N*-desmethyl compound is supposed to be oxidized by MAO A to an aldehyde.

For our investigations, we used human recombinant enzymes, namely MAO A and B, as well as the CYP enzymes most important for the metabolism of drugs, namely CYP1A2, 2C9, 2C19, 2D6, and 3A4. Detection of the amounts of the parent compound remaining after incubation and the metabolites formed was performed by HPLC and single quad MS. Initially, a C_8 column and a weakly basic mobile phase consisting of acetonitrile and aqueous $NaHCO_3$ solution were used.[22] In the course of the experiments, it became apparent that the analytes could be analyzed very well with an C_{18} column with polar endcapping using an acidic mobile phase with a high water content.

After 15 min incubation of sumatriptan with MAO A at both 10 μM and 1 μM concentrations, a small (less than 10%) but ultimately non-significant decrease in parent compound was observed (Table 1). After 60 min, this degradation was much more evident. Under these

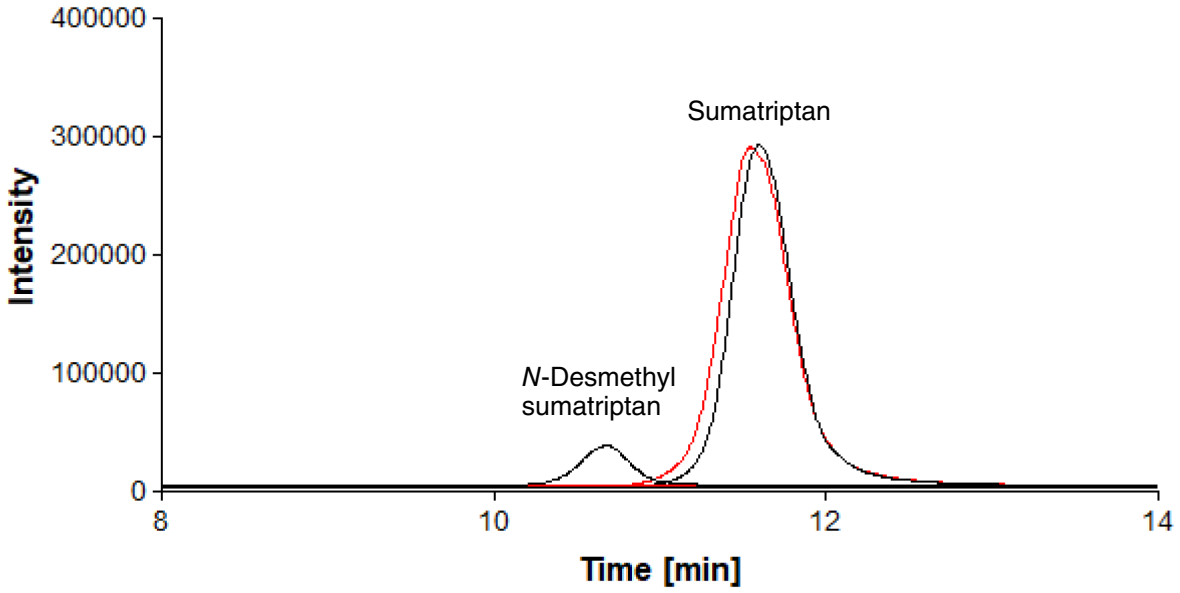

FIGURE 4 Superimposed RP-HPLC/MS chromatograms (single quadrupole, SIM, ESI+, *m/z* 296.15, 282.10, 268.10) of a sample of sumatriptan (10 µM) after 60 min incubation at 37°C with CYP2D6 (black) and an analogue prepared control sample (red); a small signal slightly larger than the noise was observed for *N*,*N*-didesmethyl sumatriptan at a retention time of 9.1 min. Chromatographic conditions: column: Accucore aQ 2.6 µm, 2.1 × 100 mm, mobile phase: acetonitrile/water/formic acid (5/95/0.1, v/v/v); flow rate: 0.2 ml/min.

TABLE 3 Conversion of *N*-desmethyl sumatriptan by different CYP enzymes

CYP Enzyme	Stability (%) of *N*-Desmethyl sumatriptan[a]	Formation (%) of *N*,*N*-Didesmethyl sumatriptan[b]
1A2	95 ± 4	3 ± 1
2C9	≥99	–
2C19	≥99	–
2D6	91 ± 2	13 ± 3
3A4	≥99	–

[a]Amount of *N*-desmethyl sumatriptan (initial concentration 10 µM) remaining after incubation at 37°C for 60 min in presence of NADPH.

[b]Quantity of *N*,*N*-didesmethyl sumatriptan formed in relation to maximum possible amount. For determination the appropriate reference compound was used.

TABLE 4 Conversion of zolmitriptan by different CYP enzymes

CYP Enzyme	Stability (%) of Zolmitriptan[a]	Formation (%) of *N*-Desmethyl zolmitriptan
1A2	89 ± 2	+
2C9	≥99	–
2C19	≥99	–
2D6	75 ± 3	+
3A4	≥99	–

[a]Amount of zolmitriptan (initial concentration 10 µM) remaining after incubation at 37°C for 60 min in presence of NADPH.

conditions, only 75%–80% of sumatriptan was still present. Much better MAO A substrates, however, were the *N*-desmethyl compound **5** and especially the *N*,*N*-didesmethyl derivative **6** (Figure 5). Of these two substances, only about 50% and 15%, respectively, were still detectable after 60 min incubation. Interestingly, similar results were obtained for the oxidative deamination of the structurally related *N*,*N*-dimethyltryptamine, *N*-methyltryptamine, and tryptamine by preparations with monoamine oxidases.[23] In contrast to MAO A, no degradation of sumatriptan could be detected in the presence of MAO B.

The oxidation product formed from sumatriptan by MAO A should be acetaldehyde **2** (Figure 1). Depending on the conversion rate, this should therefore have been present in more or less high concentration in the samples incubated with the enzyme. However, with the HPLC/MS system we used, this substance could not be detected at all. This was not surprising in a way, since aldehydes are known to be often difficult to ionize.[24-26] Moreover, aliphatic aldehydes are in equilibrium with their hydrate in aqueous solutions and therefore do not give sharp peaks in a RP chromatogram.[14] The

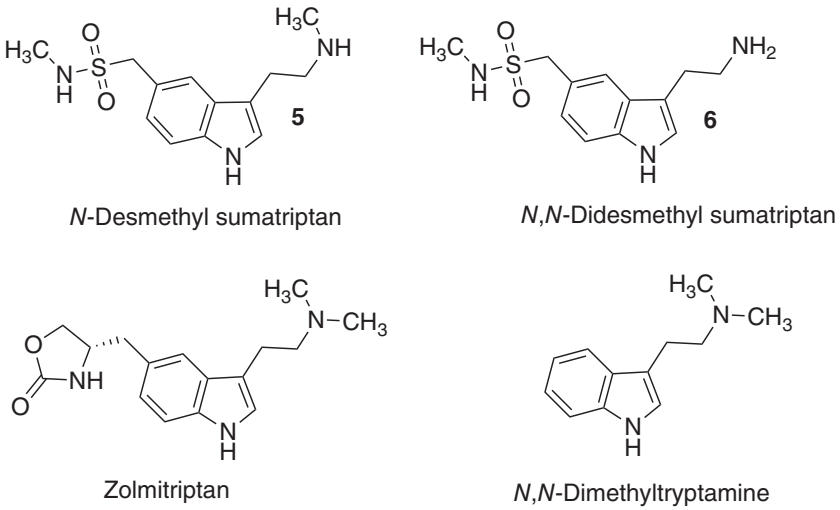

FIGURE 5 Structures of *N*-desmethyl sumatriptan, *N*,*N*-didesmethyl sumatriptan, zolmitriptan, and *N*,*N*-dimethyltryptamine.

expected aldehyde **2** should therefore be detected indirectly after derivatization. In the literature, it is described that aldehydes react with cysteine to form thiazolidine-4-carboxylic acids, which should be more ionizable.[27] For derivatization, after completion of incubation with MAO A at 37°C the samples were treated with D-cysteine, further heated to 50°C and then analyzed by HPLC/MS after the addition of acetonitrile (Figure 2). In the chromatograms obtained, the corresponding metabolism product could be clearly detected, whereby the amounts formed for the *N*-desmethyl sumatriptan derivatives were, as expected, significantly higher than for sumatriptan itself (Figure 3). In contrast to sumatriptan, no degradation was observed for zolmitriptan after incubation with MAO A and B, nor was the formation of a thiazolidine carboxylic acid derivative detectable after treatment with derivatization reagent D-cysteine. Consequently, it can be concluded that sumatriptan, but not zolmitriptan, is degraded by MAO A to an acetaldehyde derivative as described in the literature.

Next, sumatriptan was incubated with various human recombinant CYP enzymes. Previously, we had employed known specific CYP substrates to prove that the preparations used were indeed active. When sumatriptan was incubated with CYP2C9 and CYP3A4, no conversion was seen. The fact that CYP3A4 does not metabolize sumatriptan was also found in vivo.[28] On the other hand, the drug was well visibly metabolized by CYP1A2, CYP2C19, and CYP2D6. At the same time, the *N*-desmethyl derivative was detectable in all three cases in corresponding amounts and, in the incubation samples with CYP1A2 and CYP2D6, also traces of the *N*,*N*-didesmethyl derivative were found. Incubation of *N*-desmethyl sumatriptan with the selected CYP enzymes showed that it was also demethylated by CYP1A2 and CYP2D6 to form corresponding amounts of the *N*,*N*-didesmethyl derivative. A degradation by CYP2C19 as observed with sumatriptan, however, was not detectable. Interestingly, small amounts of sumatriptan *N*-oxide[16] could be identified after incubation of sumatriptan with CYP1A2 and CYP2D6. In contrast, this metabolism reaction was not observed with the other three CYP enzymes considered (CYP2C9, CYP2C19, CYP2D6).

Thus, it can be stated that sumatriptan, contrary to the general assumption in literature, can also be degraded by CYP enzymes, although the conversion rate is lower than for the specific CYP reference substrates investigated. Corresponding experiments with CYP enzymes were also carried out with zolmitriptan. As expected from the data described in the literature, this was demethylated by CYP1A2. In addition, the substance was also metabolized by CYP2D6. In both cases, the *N*-desmethyl product could be detected. However, an exact quantification of this metabolite was not carried out because we did not have the reference compound. It was striking in these experiments that sumatriptan, which according to the literature is not subject to CYP metabolism, was metabolized by the recombinant CYP1A2 and CYP2D6 enzymes to about the same extent as zolmitriptan, for which CYP metabolism was described.[12,13]

Taken together, using recombinant human enzymes, we were able to prove that sumatriptan, but not zolmitriptan, is degraded by MAO A, as described in the literature. Contrary to published assumptions,[8] we have shown that sumatriptan can also be metabolized by certain CYP enzymes in principle. The extent of in vitro metabolization of sumatriptan by these recombinant CYP enzymes is similar to that of zolmitriptan, for which CYP enzymes have been demonstrated to be involved in its metabolism.[12,13] Thus, a modified metabolic pathway for sumatriptan can be postulated (Figure 6). The question now arises whether a possible metabolization of sumatriptan by CYP enzymes has pharmacological consequences, especially with regard to drug interactions. Since sumatriptan is thought to be metabolized largely via MAO A to an indole acetic acid, concomitant use of this drug with a MAO A inhibitor such as moclobemide is considered contraindicated. Thus, the product information states that the administration of MAO A inhibitors results in a 2-fold increase in sumatriptan plasma concentration.[19] If sumatriptan is also metabolized in vivo to a considerable extent via CYP enzymes, increased plasma levels of sumatriptan could also occur with simultaneous administration of drugs which act as CYP inhibitors. In this case, the formation of *N*-desmethyl and *N*,*N*-didesmethyl metabolites of

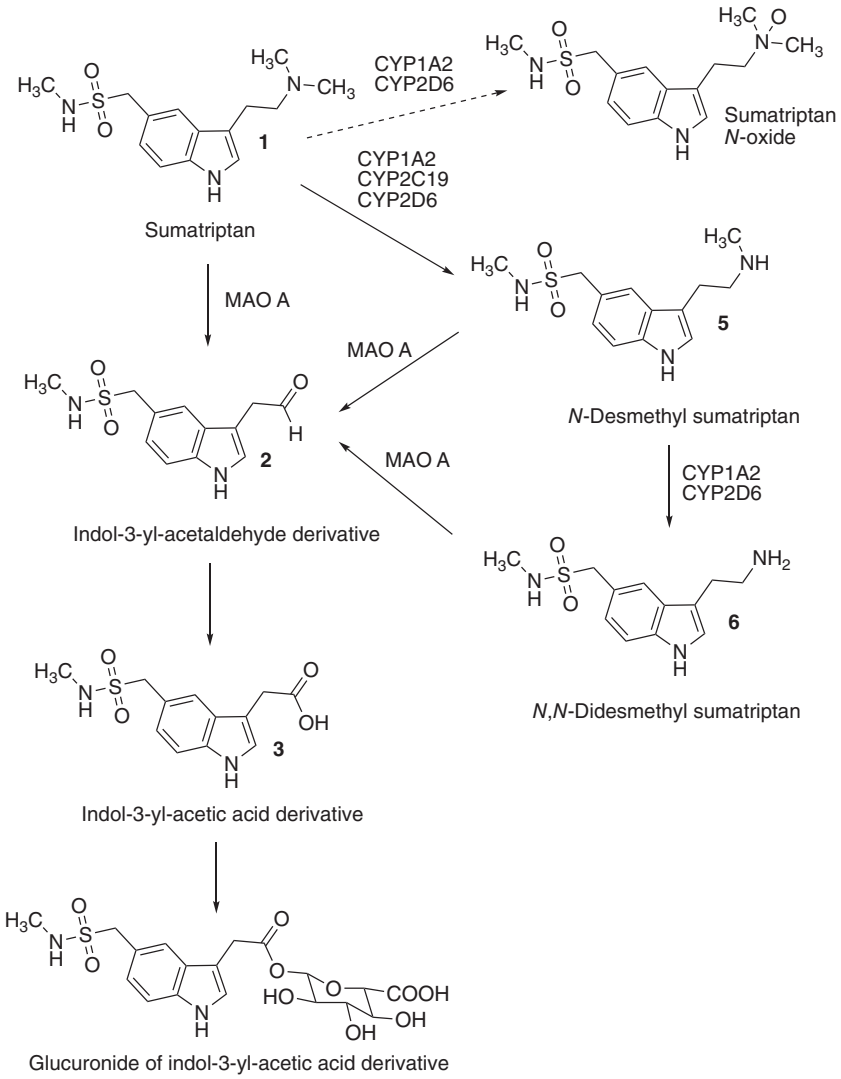

FIGURE 6 Proposed metabolic pathway of sumatriptan

sumatriptan would be inhibited. Since these metabolites are, as our experiments have shown, further degraded by MAO A deamination much faster than sumatriptan itself (Table 1), the overall degradation of sumatriptan may be slowed down by such drugs. Our studies highlight this problem, but as they are only qualitative in nature, they cannot provide a definitive answer to the relative extent of the involvement of MAO A and CYP enzymes in the metabolism of sumatriptan and to the problem of associated drug interactions. The quantitative contribution of the individual metabolic reactions to the metabolism of sumatriptan would have to be verified, for example, initially with human hepatocytes containing both mitochondrial MAO enzymes and the microsomal CYP enzymes, using specific MAO A and CYP inhibitors.

5 | NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entriesin http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[29] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[30]

AUTHOR CONTRIBUTIONS

Participated in research design: Timo Pöstges and Matthias Lehr. Conducted experiments: Timo Pöstges. Performed data analysis: Timo Pöstges and Matthias Lehr. Wrote or contributed to the writing of the manuscript: Matthias Lehr.

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DISCLOSURE

The authors declare they have no conflicts of interest with the contents of this article.

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Not applicable.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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